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Original Article

Effects of Potassium and Phosphorus Fertilizers on Arsenic Phytotoxicity and Essential Oil Yield of Two Basil Cultivars

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ABSTRACT

In this study, the effects of phosphorus and potassium fertilizer were examined on As toxicity and essential oil yeild in two basil variety. The experimental design was a factorial with two basil variety including landrace of Zabol and keshkeni luvelou as first factor, and addition of phosphorous and potassium fertilizers to soil at three levels: 50, 150 and 250 mg K kg−1 soil as second factor. Arsenic sulphate was added to all the treatments at a uniform rate equivalent to 15 mg As kg−1 soil. The experiment was conducted in 2011 at the Zabol University greenhouse in Zabol, south Iran. qualitative characteristics include the essential oil content, macronutrients and As were measured. Analysis of variance revealed that percentage of phosphorus absorbed from the soil was affected by varieties and phosphorus-potassium interaction, whereas phosphorus absorption was not significantly different across varieties. In both varieties, arsenic concentration in aerial parts reduced with increasing phosphorus addition. At different potassium fertilizer, the greatest As concentration was observed in the least potassium addition. Lowest essential oil production in both cultivars produced at the greatest As absorption.

***Keywords**: Medicinal Plants, Heavy Metals, Essential Oil, Chemical Fertilizers.*

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INTRODUCTION

Some metals are naturally found in the body and are essential to human health (iron, zinc, magnesium and copper), however, most of the heavy metals such as mercury, nickel, lead, arsenic, cadmium, aluminium, platinum, and copper (metalic form versus ionic form) act as poisonous and interference to the enzyme systems and metabolism of the body. On the other hand, some types of metal such as Cu, Mn and Zn are the natural essential components of coenzymes and they are important for growing, photosynthesis and respiration [1]. Environmental contamination with heavy metals is a serious growing problem throughout the world. In today's industrial society, there is no way to avoid the exposure to toxic chemicals and metals. Heavy metals are enriched in the environment by human activities of different kinds [2]. Results of these activities end up in outlets and wastes where they are transported to the environment by air, water or deposits, thereby increasing the metal concentrations in the environment [3]. In general, heavy metals (HM) are systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effects [4].

Arsenic is a heavy metal that is considered toxic when present in very low concentrations, on the order of parts per billion (ppb). Arsenic has been used in embalming fluids, herbicides, insecticides, defoliants, and is typically found in the vicinity of metal smelters. Arsenic is released into the environment from anthropogenic sources such as mining activities, application of arsenic-based pesticides and wood preservatives, in both inorganic and organic forms. Arsenite [As(III)] and arsenate [As(V)] are the inorganic phytoavailable forms of arsenic in soil and water. Plants vary in their sensitivity and resistance to arsenic [5]. In the developing countries heavy metal pollution becomes serious due to mining, mineral, smelting and tannery industry [6]. Heavy metal pollution not only affects the production and quality of crops, but also influences the quality of the atmosphere and water bodies, and threatens the health and life of animals and human being [7]. The use of herbal medicines has been on the rise in recent years due to their low prices and lack of awareness of people about their side effects. There is a common concept among people that herbal medicines have no side effects and that “being natural in origin, herbs are safe”. The assimilation of heavy metals in plants is obvious because of widespread heavy metals in the soil due to geo-climatic conditions [8]. Medicinal plants are the raw material for many herbal formulations and popular supplements. Heavy metals have a great tendency to accumulate in human organs over prolonged periods of time. Quality and safety standards for herbal medicine that clearly stipulate the maximum allowable value of heavy metals in herbal medicine have been enacted and put into effect by many countries [9]. However, since most of these standards are determined by referring to the quality standards for foods, they are not based on the research on herbal medicine [10]. The uptake and accumulation of heavy metals may have impacts on medicinal plants that are different from their impacts on farm crops, Therefore, it is necessary to improve quality standards for herbal medicines by examining and revising the maximum allowable values of heavy metals in medicinal plants, using research based on medicinal plants [11]. Interaction of arsenic and phosphorous in soil and its uptake by plants is complex. As and P species compounds similarities in the soil causes the soil to be competitive in their absorption. Phosphate and arsenate exhibit similar physiological behaviors and compete directly for sorption sites on soil particles [12]. As can be substituted for P in plants, but it is unable to carry out the role of P in energy transfer, the plant reacts as if there is a P deficiency. Thus, as plant Arsenic increases, the plant reacts by increasing P uptake [13]. Potassium can cause stabilization of arsenic in the soil and prevent its accumulation in the plant [14].

sweet basil (*Ocimum basilicum L.*) is an important essential oil yielding plant distributed all over India. The plant is cultivated in various agroclimatic zones, and finds extensive use as a flavouring agent, in perfumery and in pharmaceutical industry. Essential oil derived from the plant exhibits various therapeutic properties. Plant grows luxuriantly in soils contaminated with heavy metals such as cadmium (Cd), lead (Pb), copper (Cu) and arsenic (As). Plant roots take up Arsenic from soil and translocate it to aerial parts. High As concentrations in edible parts of plants pose serious health risks [15]. The objectives of this paper are: to clarify the accumulation of Arsenic in sweet basil and the influence of P and K in soil; to elucidate the responses of sweet basil to Arsenic in terms of both its growth and its yield of essential oil in sweet basil.

MATERIAL AND METHODS

Site Description

The experiment was conducted in 2011 using the greenhouse and field facilities of the University of Zabol, in Southeast Iran (61°29 N, 31°2 E, 450 m above sea level), which has a warm and arid climate with a mean annual temperature of 23 °C and average annual precipitation of 63 mm. The sandy loam soil [19% clay (<2 µm), 21% silt (2-20 µm), 41% fine sand (20-200 µm) and 19% coarse sand (200-2000 µm)], with a pH of 7.1, organic mater 1.45%, N-No3 6 ppm, P (Olsen) 12 ppm, and K 185 ppm (0-30 cm depth)] used in this study was collected from experimental agriculture farm of Zabol University. The soil was collected from the first 10cm of the soil surface, air-dried and afterwards sieved at 2mm.

Experimental Design

The experimental design was a factorial with two basil variety including local of Zabol and Keshkeni Luvelou as first factor, and addition of phosphorous and potassium fertilizers to soil at three levels: 50, 150 and 250 mg K kg^{-1} soil as second factor. Arsenic sulphate was added to all the treatments at a uniform rate equivalent to 15 mg As kg^{-1} soil. Seeds were sown in 20 cm height and 15 cm diameters pots and after that pots were placed randomly in the greenhouse, and when seedling were grown to 3 cm height plants in the pots were thinned up to five plant in each pot. During the plant growth pots were irrigated once every three days. qualitative characteristics include the essential oil content, macronutrients and Arsenic were measured.

Determination of Elements

For Arsenic analysis, dried plant tissues were ground using a ball mill and then digested with HNO3/HClO4 (3:1 [v/v]). The As concentration in digested solutions was determined by a flame atomic absorption spectrophotometer (Pu9100x-Philips). phosphorous determination using the molybdenum blue method, Phosphorous measurement was done using spectrometer (Spectrumlab 54) at 470nm [16]. Potassium concentrations were determined in all basil samples using a modified version of the method described by Zeng, Brown, and Holtz [17]. Basil leaves were ground in liquid nitrogen, dried for 48 h in a 65 _C oven, then ashed overnight in a 750 _C muffle furnace. The resulting white residue was dissolved in two 2.0 ml aliquots of 2.0 M HNO3 and after dilution with deionized water, the concentration of potassium in each sample was determined by flame atomic absorption spectrometry (Flame-Jenwy).

Statistical Analyses

Obtained data were analyzed using SAS 9.2 software and means comparison was performed based on Duncan's multiple range tests at 5% of probability level. For drawing graphs and tables EXCEL software was used.

RESULTS AND DISCUSSION

Phosphorous content (%) in shoots and roots

Analysis of variance showed that effect of variety, P and K fertilizers level combined with As and their interaction effect on P content at 1% of probability level (Table 1). P percentage in shoots and roots of bred variety was statistically higher than Zabol local variety (Table 2). Means comparison of effect of P and K fertilizers with Arsenic showed that highest P percent in roots and shoots observed at the control treatment (Table 3). Highest P content in their interaction was also observed at control treatment (Fig 1, 2).

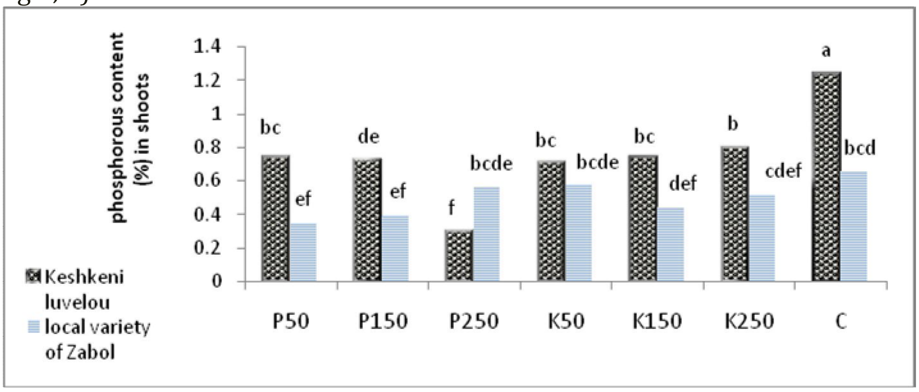

Fig 1. Interaction Varity*Fertilizers on the phosphorous content (%) in shoots.

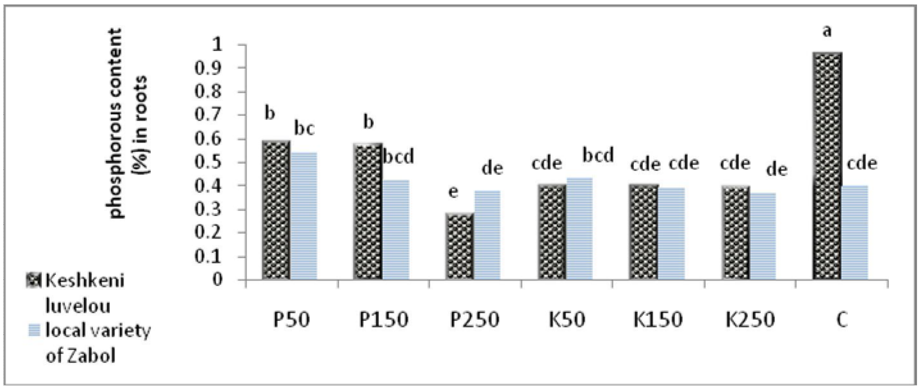

Fig 2. Interaction Varity*Fertilizers on the phosphorous content (%) in roots.

Potassium content (%) in shoots and roots

Results showed that variety effect was not significant on K percent in shoots, while its effect was significant at roots. P and K fertilizers levels with As on K content at shoots was significant at 5 and 1% probability levels, respectively. Also their interaction on K percentage of shoots and roots was significant (Table 1). There was no significant difference between roots and shoots K content (Table 2). Highest K percentage, like P %, among P and K fertilizers levels and their interaction achieved from the control treatment (Fig 3, 4).

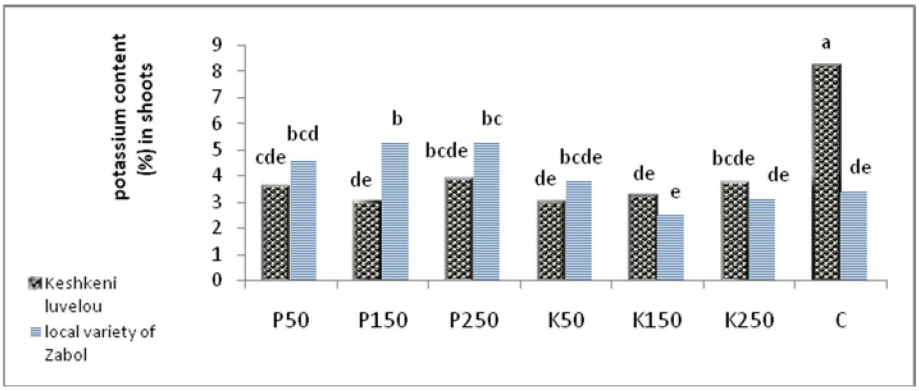

Fig 3. Interaction Varity*Fertilizers on the potassium content (%) in shoots.

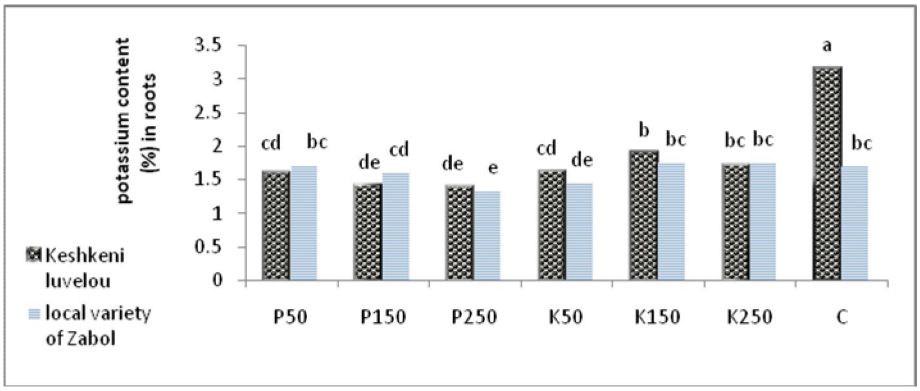

Fig 4. Interaction Varity*Fertilizers on the potassium content (%) in roots.

Arsenic concentration

Anova results showed that effect of variety and P and K fertilizers with As on Arsenic concentration was significant at 1% of probability level, while their interaction was not significant (Table 1). Means comparison of main effect of variety showed that As accumulation in shoots of Keshkeni luvelou is significantly different from local variety which may be due to its better compatibility and adoption to the studied region (Table 2). Highest and lowest Arsenic content in shoots were observed at 50 and 250 mg/kg soil P, respectively (Table 3 and Fig 5).

Being adopted with replacement of heavy metals is one of the mechanisms of heave metals toxicity alleviation by plants. Toxicity and resistance to heavy metals by plants occurs due to the interaction of nutrients like calcium, phosphorus and potassium with heavy metals. One of the main roles of this elements in the plant is to neutralize the toxic effects of heavy metals [18]. Relationship between K and Arsenic depending on whether plant is adsorbent or not, is different. In ferns (*Pteris vittata*), which is an adsorbent plant, at low levels of Arsenic, concentration of P and K due to stimulation of stems growth, increases. Also at high levels, for ionic balance, K absorption increases [19]. Another study showed that the patterns of distribution of arsenic and potassium on ferns vegetative parts are similar [20]. Research on non-absorbent (*Spatina alterniflora*) plants showed that the interaction between them, Arsenic concentration in vegetative parts reduced, which is consistent with the results of this study [21].

Similar results have also been reported in tomato [13]. Komar [22] reported a positive correlation between potassium and arsenic accumulation, and attributed the plant biomass increase to increased arsenic levels. Carbonell *et al.*, [21] during assessment of arsenic adsorption in tomato plants found that with increased uptake of this element, the incidence of injuries due to high accumulation of arsenic in the plant's reduced the ability to absorb the element. This decrease was due to roots damage which reduced its uptake and transfer from roots to shoots. In another report arsenic was found to reduce potassium absorption in tomato plants [66].

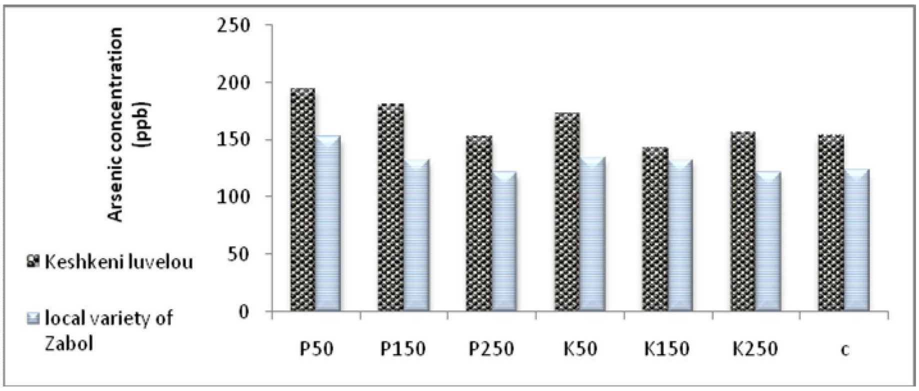

Figure 5. Interaction Varity*Fertilizers on the Arsenic concentration (ppb).

Essential oil content (%)

Results of analysis of variance showed that the effect of P and K fertilizers with Arsenic and their interactive effect on essential oil content at 1% of probability level, while variety effect was significant at 5% of probability level (Table 1). Means comparison showed significant difference of Zabol local variety and European variety for essential oil percent (Table 2). Results of means comparison of P and K fertilizers levels with Arsenic showed that P fertilizer have more effect on essential oil percentage (Table 3). Studying their interaction showed that highest essential oil percentage in both varieties produced at 150 mg/kg soil P (Fig 6).

Other research results showed that heavy metals caused an increase in reactive oxygen species and may result in cell lipid membranes. Malonil di-Aldeyde (MDA) is phenolic compound which increases with decreasing chlorogenic acid synthesis. Thus reduction of essential oil content in the peresence of heavy metals may be due to reduced phenolic precursors of essential oils [23].

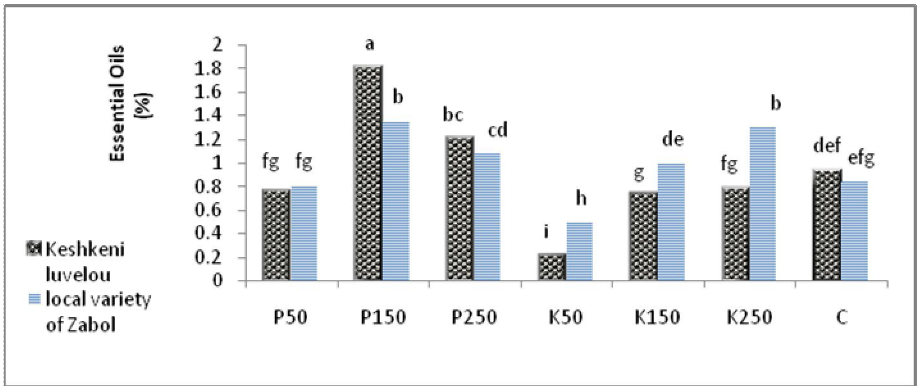

Fig 6. Interaction Varity*Fertilizers on the Essential Oils (%)

Table 1. Analysis of variance of elements on basil affected by P and K fertilizers with As.

SOV	DF	Shoots P	Roots P	Shoots K	Roots K	Arsenic	Essential oil
				Means Square			
Replication	2	0/04	0/006	11/11	0/17	192/7	0/16
Variety	1	0/72 **	0/06 **	0/09 ns	0/48 **	11857 **	0/04*
Fertilizer	6	0/19 **	0/09**	5/99 *	0/71 **	1152/6 **	0/81 **
Varity×Fertilizer	6	0/12**	0/07**	8/81 **	0/47 **	213/7 ns	0/15 **
Error	26	0/01	0/04	0/33	0/09	202/9	0/006
C.V. (%)		18/09	14/41	14/38	5/62	9/62	8/49

ns, * and ** not significant, significant at 5 and 1%, respectively.

Table 2. Basil varieties means comparison of elements

Variety	Shoots P (%)	Roots P (%)	Shoots K (%)	Roots K (%)	Arsenic (ppb)	Essential oil (%)
keshkeni luvelou	0/75 a	0/50 a	4/08 a	1/81 a	164/8 a	0/91 b
Local of Zabol	0/49 b	0/42 b	3/98 a	1/60 a	131/2 b	0/97 a

Means with similar letter are not significant at the 5% probability level

Table 3. Means comparison of elements affected by K fertilizer with As

Treatments (mg/kg)	Shoots P (%)	Roots P (%)	Shoots K (%)	Roots K (%)	Arsenic (ppb)	Essential oil (%)
P50	0/53 bc	0/55 b	4/07 bc	1/63 c	173/5 a	0/77 d
P150	0/55 bc	0/52 b	4/10 bc	1/49 d	156/4 b	1/57 a
P250	0/42 c	0/32 c	4/55 b	1/35 e	137/1 c	1/30 b
K50	0/63b	0/40 c	3/37 cd	1/52 d	153/8 bc	0/35 e
K150	0/58 b	0/39 c	2/84 d	1/81 b	137/9 bc	0/86 dc
K250	0/65 b	0/37 c	3/40 cd	1/73 bc	138/6 bc	1/04 b
control	0/99 a	0/67 a	5/89 a	2/40 a	138/7 bc	0/88 c

Means with similar letter are not significant at the 5% probability level.

CONCLUSION

Of Anova results, it was observed that P and K fertilizers can reduce the concentration of arsenic in edible parts of plants and reduce its toxicity. The interaction between P and As showed that increased applied P reduced As levels that reflects the competition between their absorption. At low P and K in soil, As uptake increased. Zabol local variety in most growth traits was better than the European one, which is due to its compatibility to studied region. Local variety absorbs less As from the soil, but had significant negative effect on growth than other treatments. Unlike the local variety, in control treatment of bred variety, As showed some simulative effect on vegetative growth.

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